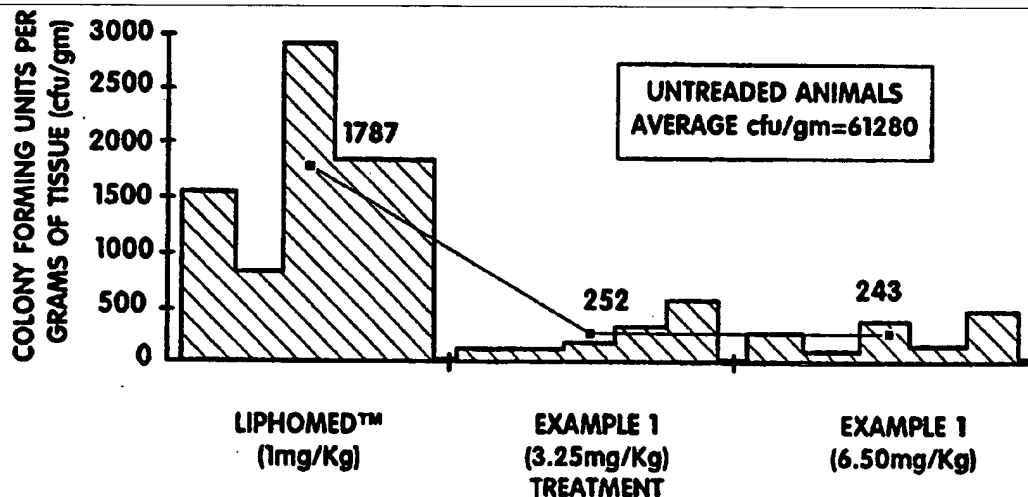




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : A61K 9/00, 9/107		A1	(11) International Publication Number: WO 94/14415
			(43) International Publication Date: 7 July 1994 (07.07.94)
(21) International Application Number: PCT/US93/10209		(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 25 October 1993 (25.10.93)			
(30) Priority Data: 07/996,661 24 December 1992 (24.12.92) US			
(71) Applicant: HEMAGEN/PFC [US/US]; 11810 Borman Drive, St. Louis, MO 63146 (US).		Published <i>With international search report.</i>	
(72) Inventors: KAUFMAN, Robert, J.; 8129 Stanford Avenue, University City, MO 63130 (US). RICHARD, Thomas, J.; 8607 Rowland Avenue, University City, MO 63132 (US).			
(74) Agents: JOSEPHIC, David, J. et al.; Wood, Herron & Evans, 2700 Carew Tower, Cincinnati, OH 45202 (US).			
(54) Title: FLUOROCARBON EMULSIONS			



(57) Abstract

Drug delivery emulsions are disclosed containing emulsified particles of a fluorocarbon, an oil in water, a surfactant and a drug solubilized in the emulsion. Compositions and methods are provided for reducing toxicity, increasing the targeting, or selectivity toward certain organs, and increasing the deposition and retention of the desired drug in these target organs, thereby increasing the efficacy of drugs.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

FLUOROCARBON EMULSIONS

TECHNICAL FIELD OF THE INVENTION

5 This invention relates to drug delivery and, in particular, to compositions and methods for reducing toxicity, increasing the targeting, or selectivity toward certain organs, and increasing the deposition and retention of the desired drug in these target
10 organs, thereby increasing the efficacy of drugs. Aqueous fluorocarbon emulsions containing an emulsified oil have been found to be surprisingly effective and safe delivery agents for drugs used in the diagnosis,
15 cure, mitigation, prevention, or treatment of disease.

BACKGROUND OF THE INVENTION

15 Drug delivery agents are needed to facilitate safe and effective use of drugs in humans and animals. For example, amphotericin B is a useful drug in the treatment of systemic fungal infection. However, its toxicity is relatively high, and effective use of the drug is often inhibited by the fact that it can exhibit

-2-

toxicity to mammalian systems. In particular, at high doses, nephrotoxicity with concomitant renal potassium and magnesium wasting is observed, K. M. Wasan, et al., *Journal of Infectious Diseases*, 161:562(1990) and references cited therein; R. Sabra, *Drug Safety*, 5(2), 94 (1990). In attempts to reduce this toxicity, a considerable effort has been expended in developing liposomal formulations of the drug in order to reduce toxicity while retaining efficacy. One of these formulations, AmBisome, is now undergoing clinical trials, O. Ringden, et al., *Journal of Antimicrobial Chemotherapy*, 28-B, 73[1991]; F. Meunier, et al., *Journal of Antimicrobial Chemotherapy*, 28-B, 83(1991). Presumably, the phosphatidylcholine based vesicles provide a harbor for the slow release of the drug, thereby attenuating its acute toxicity, while at the same time allowing it to be stored in target organs such as the liver and the spleen.

The above background with respect to amphotericin B is but an example of enormous research and development efforts to provide safe and effective methods for delivering drugs intended for use in diagnosis, cure, mitigation, treatment or prevention of disease in man or animals.

SUMMARY OF THE INVENTION

This invention is directed to drug delivery compositions that employ aqueous fluorochemical emulsions as delivery agents. The emulsions contain

-3-

emulsified particles of a fluorochemical ("PFC") and an oil in water, a surfactant and a drug solubilized in the emulsion (hereinafter sometimes "PFC/oil emulsions").

5 Surprisingly, these PFC/oil emulsions have the ability to serve as effective and safe delivery agents for very large amounts of drugs such as amphotericin B. More particularly, the toxicity of amphotericin B in rats has been ameliorated by at least
10 tenfold. Rats purposely infused with candida albicans, followed by PFC/oil emulsions containing amphotericin B, show a marked reduction in the number of candida spores found in the liver at necropsy. Other drugs such as cyclosporine, carmustine and the gadolinium
15 salt of distearyl ester of diethylenetriamine pentaacetic acid may be used.

The drug delivery PFC/oil emulsions preferably comprise an emulsified liquid PFC and a liquid fatty oil such as a triglyceride, a surfactant,
20 water and a drug solubilized in the emulsion. The term "solubilized" as used herein means the drug may be effectively suspended or dissolved in the stabilized emulsion for delivery to an animal or human.

This invention also includes methods of
25 making these emulsions containing drugs and methods of using them as drug delivery agents. Other objectives of this invention and advantages will become apparent from the following detailed description.

-4-

DETAILED DESCRIPTION OF THE INVENTION

The drug delivery emulsions of this invention comprise an oil and a fluorochemical (PFC) emulsified in water by a surfactant, and contain a solubilized drug. In general, the oil may be contained in amounts of from about 1 to 30% by weight with about 1 to 70% by volume PFC. More specifically, for instance, in medical applications for intravenous (IV) drug delivery, the preferred amount of PFC is the minimum effective amount, along with the minimum surfactant and oil, to effectively suspend the desired drug in a stable emulsion. For other uses, such as oil creams, emollients, or products designed for intramuscular, subcutaneous, or intraperitoneal injection, far greater amounts may be desirable. For IV use, about 60% v/v (115 w/v%) of PFC is a practical limit for the oil and PFC because of viscosity limitations for an intravenous product. Higher amounts may be employed for other applications. The surfactant may be contained in amounts from about 0.5 to about 10% by weight, usually about 1-2% by weight of the emulsion. Generally, the drug may be solubilized in varying amounts up to about 30%, by weight, depending upon dose, efficacy and safety requirements. The amount of any particular drug may be optimized for both the preparation of the emulsion and the concentration desired for administration. Thus, an emulsion may preferably contain a more limited amount of drug up to about 5% by

-5-

weight. For instance, a drug such as cyclosporine may be used up to about 1 or 2%, whereas an MRI imaging agent such as a gadolinium salt of distearyl ester of diethylenetriamine pentaacetic acid may be used as high
5 as about 30% or more. If desired, the emulsions may be diluted with isotonic saline, or other agents, to produce lower concentrations of the drug. These components are identified with greater particularity as follows.

10 A. Oil

The term "oil" is used herein in a general sense to identify a large class of physiologically acceptable substances whether of mineral, vegetable, animal, essential or synthetic origin. Thus, the term
15 "oil" is used herein as applied to a wide range of substances that are quite different in chemical nature.

In the classification of oils by type or function, for example mineral oil is derived from petroleum and includes aliphatic or wax-based hydrocarbons, aromatic
20 hydrocarbons or mixed aliphatic and aromatic based hydrocarbons. Also included in the mineral classification are petroleum-derived oils such as refined paraffin oil, and the like. In the vegetable classification, oils are chiefly derived from seeds or
25 nuts and include drying oils such as linseed and tung oil; semidrying such as safflower and soy bean oils; nondrying such as castor, cottonseed and coconut oils and edible soap stocks such as palm and coconut oils.

-6-

In the animal classification, oils usually occur as fats in tallow, lard and stearic acid sources. The liquid animal types include fish oils, oleic acid, sperm oil, etc. and they usually have a high fatty acid content. Included are some vegetable oils, such as olive, cottonseed, corn and peanut, as well as some special fish oils such as cod-liver, haliver, shark liver, and so forth which are used largely as medicines for their high vitamin content. A liquid fatty oil such as a mono-, di-, or triglyceride, or a mixture thereof, is the preferred oil. Medium chain triglycerides also serve as useful oils according to this invention.

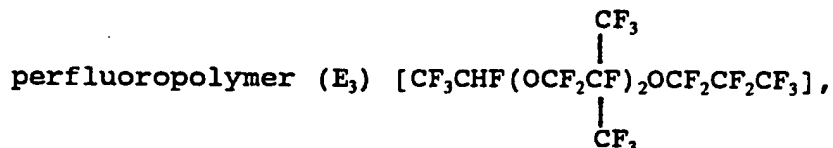
B. Fluorochemical

In this description, "fluorochemical" or "PFC" is used to describe either a highly fluorinated organic compound; a perfluorocarbon or fluorinated chemical. Further, these terms are used interchangeably. The term "perfluorocarbon" includes a "cyclic" or "acyclic" compound of carbon. Substituted derivatives thereof are also included where fluorocarbons have other elements within their structures such as oxygen, nitrogen and bromine, etc. It should also be noted that the term "perfluorocarbon" denotes substitution of all hydrogen atoms attached to the carbon atom chain or ring and any carbon side groups with fluorine. However, "fluorocarbon" is meant to include partially or substantially fluorinated

-7-

compounds. This is permissible providing that the lack of complete replacement of all hydrogens does not affect the essential non-toxic characteristics of the preferred medical fluorocarbons of this invention.

- 5 Among the perfluorocarbon compounds which may be employed are perfluorotributylamine (FC47), perfluorodimethydecalin, perfluorodecalin (PP5), perfluoromethyldecalin (PP9), perfluorooctylbromide, perfluorotetrahydrofuran (FC80),
- 10 perfluoroether (PID) $[(CF_3)_2CFOCF_2(CF_2)_2CF_2OCF(CF_3)_2]$, perfluoroether (PIID) $[(CF_3)_2CFOCF_2(CF_2)_6CF_2OCF(CF_3)_2]$,



- 15 perfluoropolymer (E₄) $[CF_3CHF)_3(OCF_2CF)_3OCF_2CF_2CF_3]$,
~~perfluoroetherpolymer (Fomblin Y/01),~~
 perfluorododecane, perfluorobicyclo[4.3.0]nonane,
 perfluorotrimethylcyclohexane, perfluorotripropylamine,
 perfluoroisopropylcyclohexane,
 20 perfluoroendotetrahydrodicyclopentadiene,
 perfluoroadamantane,
 perfluoroexotetrahydrodicyclopentadiene,
 perfluorobicyclo[5.3.0]decane,
 perfluorotetramethylcyclohexane, perfluoro-1-methyl-4-
 25 isopropylcyclohexane, perfluoro-n-butylcyclohexane,
 perfluorodimethylbicyclo[3.3.1]nonane, perfluoro-1-methyl
 adamantane, perfluoro-1-methyl-4-t-butylcyclohexane,
 perfluorodecahydroacenaphthene,

-8-

perfluorotrimethylbicyclo[3.3.1.]nonane, perfluoro-n-undecane, perfluorotetradecahydrophenanthrene, perfluoro-1,3,5,7-tetramethyladamantane, perfluorododecahydrofluorene, perfluoro-1,3-dimethyl
5 adamantane, perfluoro-n-octylcyclohexane, perfluoro-7-methyl bicyclo[4.3.0] nonane, perfluoro-p-diisopropylcyclohexane, perfluoro-m-diisopropylcyclohexane, perfluoro-15-Crown-5, and perfluoro-12-Crown-4. Chlorinated perfluorocarbons,
10 such as 1,8-dichlorooctane, bischlorobutylether, chloroadamantane and chloromethyladamantane as described in U.S. Pat. No. 4,686,024 may be used. Such compounds are described, for example, in U.S. Pats. Nos. 3,962,439; 3,493,581, 4,110,474, 4,186,253;
15 4,187,252; 4,252,827; 4,423,077; 4,443,480; 4,534,978 and 4,542,147, European Pat. Applns. Nos. 80710 and
158,996, British Pat. Specification 1,549,038 and German Offen. 2,650,586. Of course, it should be understood that mixtures of any of these highly
20 fluorinated organic compounds may also be used in the emulsions and processes of this invention.

C. Surfactant

Surfactants are needed to form stable emulsions. Any suitable surfactant may be employed
25 alone or in combination with other surfactants. For example, egg yolk phospholipids or Pluronics emulsifying agents may be used. Pluronics agents are block polymer polyols sold by Wyandotte, e.g.,

-9-

Pluronic F68, having a molecular weight of about 8,000, may be employed. Ethoxylates of cholesterol, diacyl glycerol and dialkyl ether glycerol are useful surfactants. Also, using backbones of cholesterol, diacyl glycerol or dialkyl ether glycerol, block copolymers are made by adding ethylene oxide, propylene oxide and ethylene oxide, in that order, in varying amounts to produce surfactants. In some applications for nonintravenous use, anionic or cationic surfactants may be used. The emulsions of this invention may contain alkylphosphoryl choline or alkylglycerophosphoryl choline surfactants described in Kaufman and Richard, U.S. Ser. No. 791,420, filed November 13, 1991. Specific examples of these surfactants are 1,2-dioctylglycero-3-phosphoryl choline, 1,2-ditetradecylglycero-3-phosphoryl choline, 1,2-dihexadecylglycero-3-phosphoryl choline, 1,2-dioctadecylglycero-3-phosphoryl choline, 1-hexadecyl-2-tetradecylglycero-3-phosphoryl choline, 1-octadecyl-2-tetradecylglycero-3-phosphoryl choline, 1-tetradecyl-2-octadecylglycero-3-phosphoryl choline, 1-hexadecyl-2-octadecylglycero-3-phosphoryl choline, 1-2-dioctadecylglycero-3-phosphoryl choline, 1-octadecyl-2-hexadecylglycero-3-phosphoryl choline, 1-tetradecyl-2-hexadecylglycero-3-phosphoryl choline, 2,2-ditetradecyl-1-phosphoryl choline ethane and 1-hexadecyl-tetradecylglycero-3-phosphoryl choline. Mixtures of these novel surfactants with other known

-10-

surfactants may also be employed. Anionic surfactants include alkyl or aryl sulfates, sulfonates, carboxylates or phosphates. Cationic surfactants include such as mono-, di-, tri- and tetraalkyl or aryl ammonium salts. Non-ionic surfactants include alkyl or aryl compounds, whose hydrophilic part consists of polyoxyethylene chains, sugar molecules, polyalcohol derivatives or other hydrophilic groups. Zwitter-ionic surfactants may have a combination of the above anionic or cationic groups, and whose hydrophobic part consists of any other polymer, such as polyisobutylene or polypropylene oxides. Lecithin is used in the following examples.

D. Drug

Any drug that may be solubilized in the emulsion for delivery is suitable for use in this invention. The term "drug" as used herein means any compound or composition that is intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease in man or animal (mammalian subject), as also defined by Section 201(g) of the Federal Food, Drug and Cosmetic Act. Such drugs include amphotericin B, cyclosporine, misonidazole, taxol, carmustine, camptothecin, chlorobenzoyl zidovudine, and the gadolinium salt of the distearyl ester of diethylenetriamine pentaacetic acid ("Gd-DTPA-SE"), or other lipid soluble (e.g., alkyl, alkenyl, alkynyl)

-11-

esters of this acid. Cyclosporine is a drug commonly used to prevent organ rejection after transplant. Carmustine ("BCNU") is an anti-cancer drug. Misonidazole, taxol, and other natural semi-synthetic or synthetic antineoplastic agents may be used. Gd-DTPA-SE is a drug useful in magnetic resonance imaging ("MRI"). Camptothecin is useful in the treatment of colon cancer. Zidovudine (AZT) is a popular AIDS drug and analogs such as chlorobenzoyl derivative thereof as discussed in U.S. Patent 5,041,543 are suitable for use. The invention offers a marked advantage in targeting all such drugs to RES active organs such as liver, lung, spleen and marrow, including those hydrophilic drugs made lipophilic by minor chemical modifications such as alkylation or acylation may be used. For instance, cyclosporine, which is the standard of care in liver transplant, may be delivered with the compositions of this invention. As developed above, the amount of drug in the emulsion may vary up to about 30% or more by weight, or more limited amounts up to about 5% by weight may be used.

E. Emulsion Characteristics

The emulsions of this invention are made by dispersing the above ingredients in water and homogenizing them. The surfactant enhances the dispersion and stabilization of the liquid phases. While dispersions may be generally referred to herein

-12-

as emulsions, it should be understood that they may be considered solutions, micellar solutions, microemulsions, vesicular suspensions, or mixtures of all of these physical states. The PFC may be dispersed in the oil and the oil-PFC phase emulsified in the water. However, other possible interfaces and phases are within the scope of the invention. Accordingly, the term "emulsion" as used herein covers all these states and the surfactant is employed to enhance stable mixtures of these physical states of the fluorochemical, oil, drug and water phases. For example, a fluorochemical and oil may be emulsified in water, as described in the Clark and Shaw European Pat. Appln. 87300454.3 and this application is incorporated herein by reference to describe suitable PFC/oil emulsions as drug delivery agents.

Preferably, the drug delivery emulsions of this invention contain a PFC or mixture of PFCs and most preferably contain a fluorocarbon selected from the group consisting of perfluorodecalin, perfluoromethyldecalin, perfluorodimethyladamantane, perfluorooctylbromide, perfluoro-4-methyloctahydroquinolidizine, perfluoro-N-methyldecahydroquinoline, F-methyl-1-oxa-decalin, perfluorobicyclo(5.3.0) decane, perfluorooctahydroquinolidizine, perfluoro-5,6-dihydro-5-decene and perfluoro-4,5-dihydro-4-octene.

-13-

As stated above, in general, the PFC or PFCs are used in an effective amount, along with the surfactant and oil, needed to produce an emulsion which solubilizes or effectively suspends the drug in a stable emulsion. The PFCs or mixture of PFCs may comprise about 1% to about 75% by volume, or more, of the emulsions. When the emulsions are to be used as intravenous compositions, the PFC is present in a minimum amount required to solubilize the drug in a preferred form of the invention. In a broader sense, as indicated above, the amount of oil in the emulsions may vary over a wide range of concentrations from about 0.5 to about 30% by weight, or more. It depends on the concentration and properties of the other components of the emulsion and its use. The actual oil concentration to produce an acceptable emulsion for any given set of components is determined by preparing and testing the stabilities of emulsions at various oil concentrations. Within this teaching for PFC drug delivery emulsions, for instance, about 0.5-30% by weight oil and 1-70% by volume PFC and 0.5-10% by weight surfactant are used.

The amount of a particular surfactant used in the emulsions of this invention depends upon the amounts and properties of other components of the emulsion as indicated above. Generally about 0.5-10% by weight of surfactant, preferably, about 0.5% to about 6% by weight is used. The surfactant of this

-14-

invention may be used with other surfactants as indicated above.

5 The emulsions may be prepared using any order of mixing the main components of fluorochemicals, oil, surfactant, drug and water. However, optimal emulsions may be prepared as described in the above Clark and Shaw European Appln. 87300454.3 which is incorporated herein by reference. The mixing and emulsification of components may be done using any of the conventional mixers and emulsifiers. For example, Fisher brand touch mixers, Microfluidizers, Gaulin and Rannie Homogenizers may be employed.

The following non-limiting examples illustrate various embodiments of this invention.

15 Experimental

Amphotericin B (80% purity by HPLC) used in the preparation of emulsions was purchased from Sigma Chemical of St. Louis, MO. As a control in the animal experiments, the commercially available sterile, lyophilized sodium desoxycholate formulation was prepared as directed by the Lyphomed package insert. Lecithin (Egg yolk phospholipid-proprietary intravenous grade material) was supplied by Kabi-Pharmacia of Clayton, NC. Safflower oil was obtained from California Oils, Inc. Glycerin was USP grade material, available from a variety of sources. Perfluorooctyl bromide (>99%) was received from both Atochem and Hoechst. 1,8-perfluorodichlorooctane was obtained from

-15-

3M. Na_2CO_3 (USP) was purchased from Fisher Scientific. A Millipore water purification unit supplied the water. Candida albicans (ATCC #10231) was used for all infection modeling studies.

5

Example 1

Amphotericin B (0.68g) was added to a mixture of lecithin (9.02g), safflower oil (44.13g), glycerin (9.77g), perfluorooctyl bromide (85.24g) and water (338.4g) in a Waring Blender. The blender was kept at high speed for 3-5 minutes to form a crude emulsion. This crude emulsion was then processed in a Microfluidizer homogenizer for 15 minutes at a flow rate of about 350mL/minute, while maintaining a pressure of from 7000-10,000 psig.

15

During homogenization, addition of 5% Na_2CO_3 (1.96g) controlled the pH of the mixture at about 7-9. After processing, the emulsion was poured into 100mL Wheaton serum vials and sterilized for fifteen minutes at 121°C. The composition of the resulting emulsion is expressed in the table below:

20

Table 1

Component	w/w%	w/v%	v/v%
Oil	9.02	9.79	10.64
Lecithin	1.84	2.00	2.00
Glycerin	2.00	2.17	1.72
Amphotericin	0.11	0.12	0.12
Perfluoro-octyl bromide	17.42	18.91	10.00

25

Example 2

30

Amphotericin B(2.83g) was added to a mixture of lecithin (9.01g), safflower oil (44.34g), glycerin

-16-

(10.51g), perfluorooctyl bromide (84.72g) and water (336.1g) in a Waring Blender. The blender was kept at high speed for 3-5 minutes to form a crude emulsion. This crude emulsion was then processed in a

5 Microfluidizer homogenizer for 15 minutes at a flow rate of about 350mL/minute, while maintaining a pressure of 7000-10,000 psig. During homogenization, addition of 5%Na₂CO₃ (2.35g) controlled the pH of the mixture at about 7-9. After processing, the emulsion

10 was poured into 100mL Wheaton serum vials and sterilized for fifteen minutes at 121°C. The composition of the resulting emulsion is expressed in the table below:

Table 2

15	Component	w/w%	w/v%	v/v%
	Oil	9.05	9.82	10.67
	Lecithin	1.85	1.99	1.99
	Glycerin	2.15	2.33	1.85
	Amphotericin	0.46	0.50	0.50
20	Perfluoro-octyl bromide	7.42	18.91	10.00

Example 3

Amphotericin B (3.45g) was added to a mixture of lecithin (11.12g), safflower oil (54.10g), glycerin

25 (12.43g), perfluorodichlorooctane (102.39g) and water (411.00g) in a Waring Blender. The blender was kept at high speed for 3-5 minutes to form a crude emulsion. This crude emulsion was then processed in a Microfluidizer homogenizer for 15 minutes at a flow

-17-

rate of about 350 mL/minute, while maintaining a pressure of from 7000-10,000 psig. During homogenization, addition of 5% Na₂CO₃ (2.91g) controlled the pH of the mixture at about 7-9. After processing the emulsion was poured into 100mL Wheaton serum vials and sterilized for fifteen minutes at 121°C. The composition of the resulting emulsion is expressed in the table below:

Table 3

Component	w/w%	w/v%	v/v%
Oil	9.06	9.06	10.61
Lecithin	1.86	2.01	2.01
Glycerin	2.08	2.24	1.78
Amphotericin	0.46	0.50	0.50
Perfluorodichlorooctane	17.14	18.47	10.32

The emulsions from the above examples exhibited the following physical characteristics:

Example	pH	Osmolality (mOsm)	Viscosity (centipoise)	Mean Particle Size (nM)
1	7.95	336	2.20	215
2	7.62	346	2.45	295
3	7.67	342	2.45	271

From the above data, it is clear that the emulsions are stable to sterilization.

-18-

Rodent Toxicity Studies

Sprague-Dawley rats were obtained from Sasco, Inc. (SAS:VAF/1), with an average body weight of 190-215g at time of use. Housing and environment followed the standards detailed in the "Guide for the Care and Use of Laboratory Animals". Bedding was removed/replaced and the cages were cleaned and sanitized weekly. The feeder tops were changed weekly. Each polycarbonate cage contained 5 animals, and was equipped with a Micro-Isolator hood. The hood filters were replace every other week. All rats were fed Purina rat chow and watered with fresh, chlorinated tap water ad libitum. Dosages are expressed as mg/Kg of the active ingredient.

15

Example 4

The emulsion from example 2 was infused into five randomized treatment groups of five rats each, for a total of 25 rats. The treatments were designated for 9,12,18,24 and 27mg/Kg infusions of amphotericin B. Lyphomed amphotericin B was infused at doses of 1 and 3mg/Kg. (Previous experimentation had shown that at doses of 6mg/Kg, this product was uniformly lethal within a few hours of infusion.) All animals treated with the example 2 emulsion survived and showed no adverse effects from the infusion. The Lyphomed product was safe at 1mg/Kg, but only three of five animals survived at 3mg/Kg. Thus, the improved safety

-19-

of perfluorcarbon emulsions of amphotericin is clearly established at high dose levels.

Example 5

5 This example proves the efficacy of the emulsion described in Example 1. Four groups of five animals each were infused with 3mL of a 4.9×10^6 cell/mL candida albicans suspension.

10 Thus, the total dose of candida in each animal was 1.47×10^7 cells. After four hours, the groups were treated as follows:

	Group	mg/Kg amphotericin
	Control	0.00
15	Lyphomed	1.00
	Emulsion	3.25
	Emulsion	6.50

The second group was infused with 1mg/Kg of the Lyphomed product (higher doses were not used because of the toxicity observed in Example 4). The
20 third and fourth groups were infused with the emulsion from example 2 at dosage rates of 3.25mg/Kg and 6.50 mg/Kg of amphotericin. After seven days, the animals were sacrificed and the livers were cultured to quantitate the surviving candida spores. With
25 reference to the drawing, the FIGURE summarizes the results in colony forming units per gram of tissue (cfu/g). Because of the toxicity noted in example 4 (vide supra) it was not feasible to infuse higher levels of the Lyphomed product in a single does regimen.
30

-20-

The FIGURE summarizes the results, with a bar representing the cfu/g found in the liver for each animal. The overlay chart tabulates the average cfu/g for each group. It is clear that the perfluorocarbon emulsion can deliver sufficiently high levels of amphotericin to provide effective reduction of an invasive candida infection.

We have found, by using a PFC/oil emulsion as the carrier that amphotericin B can be made less toxic so as to allow a more effective dosing regimen.

Example 6

This example is directed to an emulsion incorporating cyclosporine. The source of the cyclosporine was Sandimmune which is a concentrate for injection manufactured by Sandoz Pharmaceuticals as provided in ampoules containing 50mg of cyclosporine and 650mg of Cremophor EL (polyoxyethylated castor oil) in ethanol. Five ampoules were transferred quantitatively, using ethanol wash, to a wide mouth evaporator flask and the ethanol was removed in vacuo at a maximum temperature of 40° C.

Water (198.77g), glycerin (5.73g), lecithin (5.06g) perfluorodichlorooctane ("PFDCO") (25.22ml) were added to the flask and the mixture was pre-emulsified using the blender. The pre-emulsion was introduced into the Microfluidizer and processed, under nitrogen at a pressure of 8,000 pounds for a period of 20

-21-

minutes. During the processing, a total of 1.13g of 5% Na_2CO_3 was added to adjust the pH to approximately 8.2. The emulsion was bottled in 100 ml serum bottles. The bottles were not sterilized. The emulsion of this example contains 2.01 w/v% lecithin, 6.44 w/v% polyoxyethylated castor oil, 10 v/v% PFDCO and 0.5 w/v% cyclosporine. Other physical properties of the emulsion include a pH at 25° C of 8.10; 346mOsm osmolality; 2.25 centipoises viscosity at 37° C; and 158nM Brookhaven PSD.

Example 7

This example is directed to the preparation of an emulsion incorporating a gadolinium salt of distearyl ester of diethylenetriamine pentaacetic acid ("Gd-DTPA-SE"). First a co-mixture of lecithin and Gd-DTPA-SE was made by stirring a solution of 18.4g of Gd-DTPA-SE in 200 ml of chloroform and adding dropwise a solution of 6.0g of lecithin in 100ml chloroform. The chloroform was removed in vacuo at a maximum temperature of 30° C and the resulting solid was dried in vacuo for sixteen hours. The emulsion was then prepared by combining water (194.8g), glycerin (5.86g), safflower oil (4.91g), the above prepared co-mixture (20.10g), and perfluorodichlorooctane (PFDCO) (24.84g), and pre-emulsifying the mixture using a Waring Blender. The pre-emulsion was introduced into a Microfluidizer and processed, under nitrogen, at a pressure of 7,000

-22-

to 8,000 psig for a period of 40 minutes. During the processing, 5% Na₂CO₃ (15.90g) was added by syringe to hold the pH at about 8. The emulsions were then bottled in 100ml serum bottles which were then either sterilized at 121° C for 15 minutes or not sterilized. The emulsion composition resulting from these procedures contains 1.90 w/v% lecithin, 1.85 w/v% safflower oil, 9.35 v/v% PFDCO, and 5.67 w/v% Gd-DTPA-SE. For the emulsion that was not sterilized the other physical properties include a pH at 25°C of 7.83; 367mOsm osmolality; 1.69 centipoises viscosity at 37°C; and 184nM Brookhaven PSD. For the emulsion that was sterilized the other physical properties include a pH at 25°C of 4.91; 352mOsm osmolality; 2.45 centipoises viscosity at 37°, and 143nM Brookhaven PSD.

Example 8

This example is directed to an emulsion incorporating carmustine. A co-mixture of lecithin and carmustine was prepared by adding the equivalent of 1.5g of sterile carmustine of Bristol Laboratories into a wide mouth evaporator flask with the supplied ethanol. Lecithin (6g) was dissolved in 100ml of ethanol and added to the carmustine solution. The alcohol was removed in vacuo at a maximum temperature of 25°C. The resulting sticky solid was dried in vacuo for three hours. The emulsion was then prepared by adding water (225g), glycerin (6.7g), safflower oil

-23-

(29.48g) and PFDCO (30.55 ml) into the flask containing the carmustine and the mixture was pre-emulsified using a blender. The pre-emulsion was introduced into the Microfluidizer and processed, under nitrogen, at a pressure of 8,000 pounds for a period of 18 minutes. The emulsion temperature was held below 22°C throughout the processing by an ice bath. The emulsion was then bottled in 100ml serum bottles without sterilization. The emulsion of this example contains 2.00 w/v% lecithin, 9.81 w/v% oil, 10.17 v/v% PFDCO and 0.50 w/v% carmustine. Other physical properties of the emulsion include a pH at 25°C of 3.93; 335mOsm osmolality; 2.20 centipoises viscosity at 37°C; and 298nM Brookhaven PSD.

15

Example 9

Employing procedures similar to Example 8, camptothecin emulsions were successfully prepared.

Example 10

1-[3-Azido-5-O-(3-chlorobenzoyl)-2,3-dideoxy- β -D-erythropentofuranosyl]-5-methyl-2(1H)-pyrimidinone (also "chlorobenzoyl zidovudine") was made by the procedure outlined in U.S. Pat. 5,041,543. This compound (1.25g) was mixed with egg yolk phospholipid supplied by Kabi-Pharmacia (10.0g) and the resulting mixture was dissolved in chloroform. The chloroform was evaporated, leaving a waxy residue. To the residue

-24-

was added water (182.3g), safflower oil (25.0g), glycerin (5.69g), 5% sodium carbonate solution (1.42g) and 1,8-perfluorodichlorooctane (PFDCO) (44.32g). This entire mixture was agitated briefly with a high speed
5 blender. After approximately 2 minutes, a crude emulsion formed. This crude emulsion was fed to the Microfluidizer and processed, under nitrogen, at a high pressure (7500 psig) for about 15 minutes. The resulting emulsion had a pH of 7.32, an osmolarity of
10 340 mOsm, a viscosity of 2.45 centipoises, and a mean particle size of 173 nanometers. HPLC analysis of the product emulsion proved that the prodrug derivative remained intact during emulsification, with no detectable hydrolysis to the parent AZT. The
15 concentration of prodrug emulsified was thus assayed at 0.42%.

In view of the above description, other modifications may be made as one of ordinary skill in this art will understand without departing from the
20 scope of this invention.

-25-

Claims are;

1. A physiologically acceptable drug delivery emulsion comprising emulsified particles of a fluorochemical and an oil in water, a surfactant and a drug solubilized in the emulsion.

2. The drug delivery emulsion of claim 1 wherein the fluorochemical is a perfluorocarbon.

3. The drug delivery emulsion of claim 1 wherein the fluorochemical is selected from the group consisting of perfluorodecalin, perfluoromethyldecalin, perfluorodimethyldecalin, perfluorodimethyladamantane, perfluorooctylbromide, perfluoro-4-methyl-
5 octahydroquinolidizine, perfluoro-N-methyldecahydroquinoline, F-methyl-1-oxa-decalin, ~~perfluorobicyclo (5.3.0) decane~~, perfluorooctahydroquinolidizine, perfluoro-5,-6-
10 dihydro-5-decene, perfluoro-4,-5-dihydro-4-octene, 1,8-perfluorodichlorooctane, perfluorobischlorobutylether, perfluoro-15-Crown-5, and perfluoro-12-Crown-4, and mixtures thereof.

4. The drug delivery emulsion of claim 1 wherein said emulsion is stable after heat sterilization.

-26-

5. The drug delivery emulsion of claim 1 wherein a liquid fatty oil is present in an amount of about 0.5 to about 30% by weight of the emulsion.

6. The drug delivery emulsion of claim 5 wherein the oil is selected from the group consisting of mono-, di-, and triglycerides, and mixtures thereof.

7. The drug delivery emulsion of claim 1 wherein the surfactant is present in an amount of from about 0.5 to about 10% by weight of the emulsion.

8. The drug delivery emulsion of claim 7 wherein the surfactant is present in an amount of from about 1 to about 6% by weight of the emulsion.

9. The drug delivery emulsion of claim 1 wherein the fluorochemical is present in an amount of from about 1 to about 75% by volume of the emulsion.

10. The drug delivery emulsion of claim 9 wherein a surfactant is contained in amount of from about 0.5 to about 10% by weight of the emulsion.

11. The drug delivery emulsion of claim 10 wherein said fluorochemical is contained in an amount of at least about 40% by volume of the emulsion.

-27-

12. The drug delivery emulsion of claim 1 wherein said drug is contained in an amount of up to about 30% by weight of the emulsion.

13. The drug delivery emulsion of claim 12 wherein said drug is contained in an amount of up to about 5% by weight of the emulsion.

14. The drug delivery emulsion of claim 1 wherein the drug is selected from the group of amphotericin B, cyclosporine, gadolinium salt of a lipid soluble ester of diethylenetriamine pentaacetic acid, misonidazole, taxol, camptothecin, chlorobenzoyl zidovudine and carmustine.

15. The drug delivery emulsion of claim 1 wherein said drug is amphotericin B.

-28-

16. A physiologically acceptable drug delivery emulsion comprising emulsified particles of a fluorochemical selected from the group consisting of perfluorodecalin, perfluoromethyldecalin, perfluorodimethyldecalin, perfluorodimethyladamantane, perfluorooctylbromide, perfluoro-4-methyloctahydroquinolidizine, perfluoro-N-methyldecahydroquinoline, F-methyl-1-oxa-decalin, perfluorobicyclo (5.3.0) decane, perfluorooctahydroquinolidizine, perfluoro-5,-6-dihydro-5-decene, perfluoro-4,-5-dihydro-4-octene, 1,8-perfluorodichlorooctane, perfluorobischlorobutylether, perfluoro-15-Crown-5, and perfluoro-12-Crown-4, and mixtures thereof, and an oil in water, a surfactant and a drug solubilized in the emulsion, said fluorochemical is contained in an amount from about 1 to 75% by volume of the emulsion and wherein the emulsion is stable after heat sterilization.

17. The drug delivery emulsion of claim 13 wherein said oil is a liquid fatty oil present in amount of about 0.5 to about 30% by weight of the emulsion.

18. The drug delivery emulsion of claim 17 wherein the oil is selected from the group consisting of mono-, di-, and triglycerides, and mixtures thereof.

-29-

19. The drug delivery emulsion of claim 16 wherein said drug is contained in an amount up to about 30% by weight.

20. The drug delivery emulsion of claim 19 wherein the drug is selected from the group of amphotericin B, cyclosporine, gadolinium salt of distearyl ester of diethyleneetriamine pentaacetic acid
5) misonidazole, taxol, camptothecin, chlorobenzoyl zidovudine and carmustine.

21. A method for diagnosing, curing, mitigating, preventing or treating a disease in a mammal by administering to said mammal an effective amount of the emulsion of claim 1.

22. A method for diagnosing, curing, mitigating, preventing or treating a disease in a mammal by intravenously administering to said mammal an effective amount of the emulsion of claim 14.

23. A method for diagnosing, curing, mitigating, preventing or treating a disease in a mammal by administering to said mammal an effective amount of the emulsion of claim 15.

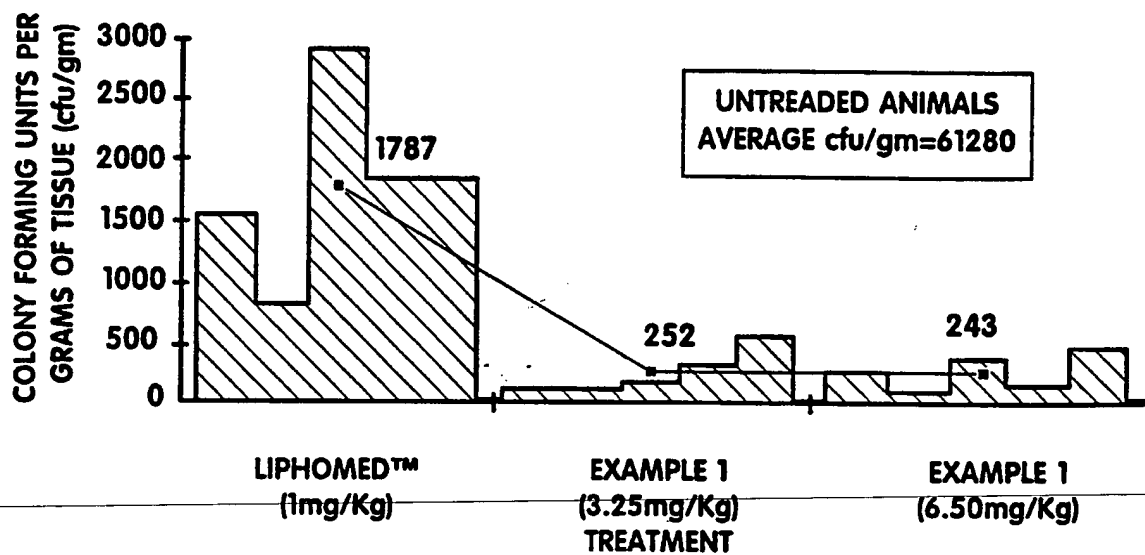
-30-

24. A method for diagnosing, curing, mitigating, preventing or treating a disease in a mammal by intravenously administering to said mammal an effective amount of the emulsion of claim 16.

25. A method for diagnosing, curing, mitigating, preventing or treating a disease in a mammal by administering to said mammal an effective amount of the emulsion of claim 18.

26. A method for diagnosing, curing, mitigating, preventing or treating a disease in a mammal by administering to said mammal an effective amount of the emulsion of claim 20.

1/1



SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 93/10209

A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 A61K9/00 A61K9/107

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHEMICAL ABSTRACTS, vol. 101, no. 22, 26 November 1984, Columbus, Ohio, US; abstract no. 198210p, 'perfluoro compds. emulsions for tumor chemotherapy' see abstract & JP,A,59 130 813 (GREEN CROSS CORP) 27 July 1984	1-3, 5-14,21, 22,25
Y	EP,A,0 399 842 (THE GREEN CROSS CORP.) 28 November 1990 see claims 1,4 see page 3, line 33 - page 4, line 13 see page 5, line 13 - line 16 --- -/--	1-3, 7-14,21, 22,25

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

26 January 1994

Date of mailing of the international search report

04.02.94

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer

Ventura Amat, A

INTERNATIONAL SEARCH REPORT

In International Application No

PCT/US 93/10209

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>WO,A,89 10118 (HEMAGEN/PFC) 2 November 1989 see claims 1-8 see page 11, line 26 - page 14, line 20 see page 16, line 6 - line 13 -----</p>	5,6

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 93/ 10209

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
REMARK: Although claims 21-26 are directed to a method of treatment of the animalbody, the search has been carried out and based on the alleged effects of the composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 93/10209

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP-A-59130813	27-07-84	NONE	
EP-A-0399842	28-11-90	CA-A- 2017542 JP-A- 3072423	26-11-90 27-03-91
WO-A-8910118	02-11-89	US-A- 5171755 AU-B- 629832 AU-A- 3566489 EP-A- 0449825 JP-T- 3502693 OA-A- 9322	15-12-92 15-10-92 24-11-89 09-10-91 20-06-91 15-09-92